Claims

1. Method for the direct sequencing of a DNA molecule from a complex mixture of nucleic acids in which truncated as well as DNA molecules of full length are simultaneously synthesized between two positions on the said DNA molecule in a thermocyclic reaction which initially comprises a DNA molecule, a first primer, a second primer, a reaction buffer, a thermostable DNA polymerase, deoxynucleotides or derivatives thereof and a dideoxynucleotide or another terminating nucleotide, wherein the thermostable DNA polymerase has a reduced discrimination against the four ddNTPs compared to wild-type Taq polymerase.

2. Method for the direct sequencing of a DNA molecule in which truncated DNA molecules as well as DNA molecules of full length are simultaneously synthesized between two positions on the said DNA molecule in a thermocycling reaction which initially comprises a DNA molecule, a first primer, a second primer, a reaction buffer, a thermostable DNA polymerase, deoxynucleotides or derivatives thereof and a dideoxynucleotide or derivatives thereof, wherein the initial ratio of the said primers to one another is larger than 1 in the said thermocycling reaction.

3. Method as claimed in claims 1 and 2, wherein the ratio of the said primers is about 2:1.

4. Method as claimed in claims 1 to 3, wherein the said method is carried out in a single step, in a single container, vessel or tube.

5. Method as claimed in one of the claims 1 to 4, wherein the said primer has a length of at least 25 nucleotides.

6. Method as claimed in every claim 1 to 5, wherein the said first primer is labelled.

7. Method as claimed in one of the claims 1 to 6, wherein the said first primer and said second primer are labelled differently.

8. Method as claimed in one of the claims 1 to 7, wherein the thermocycling reaction additionally contains a thermostable pyrophosphatase.

9. Method as claimed in one of the claims 1 to 8, wherein the annealing and synthesis steps of the thermocycling reaction are carried out at a temperature of at least about 62°C.

10. Method as claimed in one of the claims 1 to 9, wherein said DNA molecule is genomic DNA.

11. Method as claimed in claim 10, wherein the said genomic DNA is larger than or equal to 2 kb in length.

12. Method as claimed in one of the claims 1 to 11, wherein the source of the nucleic acid molecules to be sequenced is a source selected from body fluids such as sperm, urine, blood or blood samples, hairs, a single cell, cells or fractions thereof, tissue or fractions thereof and tissue cultures.

13. Method as claimed in one of the claims 2 to 12, wherein the said thermostable polymerase has a reduced discrimination against the 4 ddNTPs compared to wild-type Taq DNA polymerase in the buffer or under the conditions that are used for thermocycling.

14. Method as claimed in one of the claims 1 to 13, wherein the said thermostable polymerase is a Taq DNA polymerase with a Tabor-Richardson mutation which also has no 5'-3' exonnuclease activity or a functional derivative thereof.

15. Method as claimed in one of the claims 1 to 14, wherein the said thermostable polymerase is Taq DNA polymerase (-exo5'-3')(F667Y) or a functional derivative thereof.

16. Use of the method as claimed in one of the claims 1 to 15 for the determination of a sequence of a nucleic acid.

17. Use of the method as claimed in one of the claims 1 to 15 for the direct sequencing of eukaryotic genomic DNA.

18. Use of the method as claimed in one of the claims 1 to 15 for the direct sequencing of human chromosomal or mitochondrial DNA.

19. Use of the method as claimed in one of the claims 1 to 15 for the direct sequencing of human RNA.

20. Use of the method as claimed in one of the claims 1 to 15 for the direct sequencing of unpurified plasmid DNA from bacterial colonies.

21. Use of the method as claimed in one of the claims 1 to 15 for the direct sequencing of unpurified single-stranded or double-stranded DNA from bacteriophages.

22. Kit for the direct sequencing of a nucleic acid molecule from a complex mixture of nucleic acids containing a reaction buffer, deoxynucleotides or derivatives thereof and a dideoxynucleotide or another terminating nucleotide and a thermostable polymerase which has a reduced discrimination against ddNTPs compared to wild-type Taq DNA polymerase.

23. Kit for the direct sequencing of a nucleic acid molecule containing a reaction buffer, deoxynucleotides or derivatives thereof, dideoxy-nucleotides or another terminating nucleotide, a thermostable polymerase and two primers whose ratio is larger than 1.

24. Kit for sequencing a nucleic acid molecule as claimed in claim 23, wherein the thermostable polymerase has a reduced discrimination against ddNTPs compared to wild-type Taq DNA polymerase.